Vaccination against autoimmune encephalomyelitis with T-lymphocite line cells reactive against myelin basic protein

Avraham Ben-Nun*, Hartmut Wekerle† & Irun R. Cohen*

*Department of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel
†Max-Planck-Institut für Immunbiologie, Freiburg, FRG

Despite differences in initiating events and pathophysiology, the aetiological agents of all autoimmune diseases are lymphocytes specifically reactive against normal constituents of the individual. Recently we have isolated and grown as a cell line rat T lymphocytes reactive against myelin basic protein (BP)[1]. This T-cell line originated from rats in which we had induced experimental autoimmune encephalomyelitis (EAE) by immunizing them against BP. Inoculation of syngeneic rats with the T-cell line led to the relatively rapid onset of EAE[1]. We report here that attenuation of this cell line provides an agent for establishing resistance to induction of active EAE. Intravenous (i.v.) inoculation of syngeneic rats with cells of the line attenuated by treatment with irradiation or mitomycin C augmented resistance to EAE caused by an encephalitogenic challenge with BP. Thus, aetiological agents of autoimmune disease, like those of microbial disease, when suitably attenuated can be used as effective vaccines.

EAE can be induced in susceptible animals such as rats, guinea pigs, rabbits, monkeys[2] or man[3] by injecting them with BP emulsified in an adjuvant such as complete Freund's adjuvant (CFA). In Lewis rats the disease is characterized by paralysis that is most marked in the tail and hind limbs and which starts usually ~12 days after a single injection of BP in CFA. Histologically the central nervous system shows perivascular infiltrates of mononuclear cells[4]. Unless the rats are aged or have undergone splenectomy or thymectomy[5] they recover spontaneously from clinical paralysis after a number of days. To study the pathophysiology of EAE we have isolated and propagated *in vitro* a line of Lewis rat T lymphocytes that reacts only against BP, designated Z1a (ref. 1). We found that i.v. inoculation of as few as 10^5 cells of the Z1a line led to the onset of paralysis in ~4 days. Inoculation of 10^6 or more cells produced paralysis in ~2–3 days. Most rats recovered from this form of EAE if properly nursed during their paralysis. Table 1 shows the specificity of the proliferative response of the anti-BP Z1a line compared with that of the Z1c line which had been selected for its reactivity against another antigen, the purified protein derivative (PPD) of the mycobacteria present in CFA. The cells of each line responded to its specific antigen, and were also activated by the T-cell mitogen concanavalin A (Con A). Essentially all the cells in both the Z1a and Z1c lines proved to be positive for a T-cell marker using a specific monoclonal antibody (Sera-lab, UK; clone W3/13 HLK) in an immunofluorescence assay[6].

We investigated the effect of attenuating the Z1a line by inhibiting its cell division. Table 2 shows that i.v. injection of 1×10^7 untreated cells of the Z1a line into syngeneic Lewis rats produced EAE in 18 of 20 rats within 2–3 days. Irradiation of the cells with 1,500 rad or treatment with mitomycin C, agents that block cell division, abrogated the ability of these cells to cause EAE. None of 25 rats that received Z1a cells treated in this way developed EAE. Furthermore, inoculation of

Table 1 Anti-BP and anti-PPD T-cell lines are immunospeci

T-cell line	Proliferative response (c.p.m. $\times 10^{-3} \pm$ s.d.) No antigen	BP	PPD	Con
Anti-BP (Z1a)	1.7±0.3	48.7±6.1	1.9±0.4	71.4±9
Anti-PPD (Z1c)	1.4±0.7	1.2±0.4	77.9±10.4	82.3±12

The Z1a and Z1c cell lines originated from the same draining ly node cell population obtained from female Lewis rats immunized BP in CFA as described elsewhere[1]. To develop the cell lines, Lewis were injected in each footpad with 0.05 ml containing BP (25 extracted from guinea pig spinal cords[10] emulsified in equal volume phosphate-buffered saline and CFA containing 4 mg ml^{-1} of *Mycob terium tuberculosis* $H_{37}Ra$ (Difco). On day 9, the draining lymph no were removed and a single-cell suspension prepared. The cells were t selected *in vitro* for BP or PPD by culturing them with either antigen 72 h. The lymphoblasts that were generated were separated by a continuous Ficoll gradient and propagated and maintained *in vitro* cell line for several months in medium enriched with T-cell growth fa as reported elsewhere[1]. The proliferative responses of the T-cell lin were tested *in vitro* as follows[1]. Briefly, 2.5×10^4 cells of either Z1 Z1c cells were cultured in quadruplicates in flat-bottom microtitre w in 0.2 ml of Eagle's medium supplemented with 1% fresh autologous serum, 2-mercaptoethanol (5×10^{-5} M), L-glutamine (2×10^{-3} M) antibiotics (streptomycin and penicillin) with added irradia (1,500rad) normal syngeneic lymph node cells as accessory cells ($5 \times$ cells ml^{-1}) and antigens, BP (50 μg ml^{-1}), or PPD (25 μg ml^{-1}; Sta Serum Institut) or Con A (2.5 μg ml^{-1}; Miles-Yeda, Israel). After 2 the cultures were pulsed with ^{3}H-thymidine (1 μCi per well, spec activity 10 Ci mmol^{-1}; Nuclear Research Centre, Israel) for 16 h. T cells were then collected on glass fibres using an automatic collector a thymidine incorporation measured in a liquid scintillation counter.

untreated cells of the Z1c line also failed to induce EAE. Thi induction of EAE is a function of the specific anti-BP Z1a line property lost after irradiation or treatment with mitomycin

We then tested whether inoculation with cells incapable inducing EAE could affect the susceptibility of rats to acti induction of EAE by later challenge with BP in CFA. Table shows that untreated Lewis rats were highly susceptible induction of EAE on injection with BP in CFA; 69 of 71 r developed disease. Intravenous inoculation of cells of the Z anti-PPD line, either untreated or irradiated, did not affect t susceptibility and EAE was induced in all 20 rats challen with BP in CFA. In contrast, a single i.v. injection of 1×10^7 Z cells attenuated by treatment with mitomycin C or irradiati led to significantly increased resistance to induction of EA Only 14 of a total of 40 rats showed any signs of paralysis and t degree of the paralysis in these rats was judged to be mu milder than that appearing in the other groups. Thus it see that vaccination with attenuated autoimmune T lymphocyt

Table 2 Attenuated T lymphocytes of the Z1a anti-BP line do n produce EAE

Line	Inoculation of T-cell lines Treatment	Incidence of EAE
Anti-BP (Z1a)	Untreated	18/20
	Irradiated	0/15
	Mitomycin C	0/10
Anti-PPD (Z1c)	Untreated	0/20

Healthy female Lewis rats (2–3 months old) were injected i.v. wi 1×10^7 cells of T-lymphoblast cell lines specifically reactive against B (Z1a) or PPD (Z1c). Before inoculating the cell lines into norm syngeneic animals, they were re-stimulated *in vitro* with the relevan antigen, in the presence of irradiated (1,500 rad) syngeneic access cells for 72 h (ref. 1). The cells, >80% lymphoblasts, were then collect and injected, either untreated or attenuated by irradiation (1,500 ra from a ^{60}Co source, or treatment with mitomycin C (50 μg per 10^7 cel per ml; Sigma) at 37°C for 40 min. The treated cells were wash extensively before being inoculated. EAE was diagnosed clinically overt paralysis of the hind limbs and histologically by perivascul mononuclear cell infiltration of the central nervous system[4].

0028-0836/81/270060—02$01.00

...ated anti-BP T-cell line vaccinates rats against induction of EAE

	Incidence of EAE in response to injection of BP in CFA	% Inhibition of EAE
	69/71	—
	10/10	0
	10/10	0
	8/25*	68
	6/15*	60

...duced in naive Lewis rats (2–3 months old) or in animals ...vaccinated i.v. 3 weeks earlier with 10^7 cells of the ...T-cell line or with cells of the anti-BP Z1a T-cell line that ...irradiated (1,500 rad) or treated with mitomycin C, as ...Table 2 legend. EAE was induced by injecting BP in CFA ...footpads of the animals, as described in Table 1 legend.

...protection against active EAE for about 65% of the

...do not know the mechanism by which the attenuated T ...ates increased resistance to induction of EAE; ...t seems reasonable to suspect that some process of ...was involved. The Z1a anti-BP lymphocytes probably ...from the ineffective Z1c anti-PPD lymphocytes in the ...their antigen receptors (Table 1). Antigen receptors ...phocytes[7] as well as of B lymphocytes or antibodies[8] can ...nogenic. Immunity against antigen receptors, anti-...mmunity, has been proposed to serve as a mechanism ...ulates immune responses by suppressing or activating ...clones of lymphocytes bearing the target receptors[9]. ...tion with attenuated Z1a cells might have produced an ...response against endogenous clones of lymphocytes ...BP receptors. As anti-BP clones are the aetiological ...EAE, development of the disease would be inhibited. ...our results could be explained by anti-receptor ...y raised against the autoimmune lymphocytes that ...EAE. However, other explanations are possible and ...receptor hypothesis must be tested experimentally.

...ever the mechanism of protection, the procedure ...ed here can be conceptually related to vaccination ...infectious diseases in which inoculation of an attenuated ...of disease induces a degree of protection against the ...pathogen. In the case of autoimmunity, the aetiological ...of disease is not a microbe, but arises within the immune ...of the individual. Our results indicate that an artificially ...autoimmune disease may be mitigated or prevented by ...tion against specific effector lymphocytes. A different ...ulated problem is posed by the need to treat the spon-...s often chronic processes that characterize the important ...mmune diseases of man.

...thank Mr H. Otmy for technical assistance and Professor ...dman for his support. This study was supported by a grant ...the Stiftung Volkswagenwerk, Hannover.

...February; accepted 30 March 1981.

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...0161—03$01.00

C-terminal sequence of the secreted form of mouse IgD heavy chain

Renate Dildrop & Konrad Beyreuther

Institut für Genetik der Universität zu Köln, D-5000 Köln, 41, FRG

Immunoglobulins have been identified as membrane-bound molecules on the surface of B lymphocytes and as secreted products of plasma cells. In the case of immunoglobulin M(IgM) the carboxy-terminal sequences of the μ-chains of membrane-bound and secreted molecules differ from each other and are encoded by different exons of the μ constant region (Cμ) gene. The coding sequence for the C-terminus of the secreted μ-chain is contiguous with the 3′ end of the Cμ4 exon and separate exons downstream of Cμ4 encode the C-terminus of the membrane-bound chain[1–3]. Immunoglobulin D is also found membrane-bound and as a secreted molecule, and recent data indicate that the exon arrangement of the Cδ gene is in part similar to that of the Cμ gene[4,5]. However, the amino acid sequence analysis presented here demonstrates that in the case of IgD the C-terminus of the secreted δ-chain is encoded by a separate exon (the CδDC exon of Tucker *et al.*[5]) and not by the CδAC sequence which corresponds topographically to the sequence expressed at the C-terminus of secreted μ chains.

The cell line B1-8.δ1 (IgD, λ1) has been isolated as a switch variant of the cell line B1-8.64.1 (IgM, λ1) and is of C57BL/6 origin[6]. It secretes a monoclonal IgD antibody with specificities for the hapten (4-hydroxy-3-nitrophenyl)acetyl (NP). Anti-NP antibodies from B1-8.δ1 ascites fluid were purified by affinity chromatography[6]. The heavy chains of these molecules are linked to each other and to the light chains by disulphide bridges (ref. 6 and unpublished data). After complete reduction and carboxyamidomethylation the heavy and light chains were eluted from Sephadex G-100 with 4.5 M urea, 1 M propionic

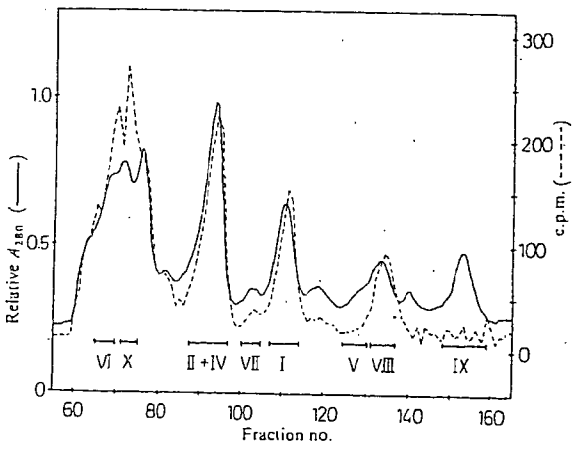

Fig. 1 Gel filtration of cyanogen bromide-cleaved peptides of ^{14}C-carboxyamidomethylated B1-8.δ1 heavy chain. Cyanogen bromide cleavage of 50 mg of the completely reduced and alkylated[9] heavy chain was performed in 70% formic acid at a concentration of 10 mg ml^{-1} with a fivefold excess (w/w) of CNBr for 24 h at 20 °C. The mixture was dried under a stream of nitrogen. The peptides were dissolved in 3.5 ml of 0.1 M formic acid containing 6 M deionized urea and applied to a Sephadex G-50 superfine column (2 × 200 cm) equilibrated in the same solvent. Fractions of 3.3 ml were collected and aliquots of 20 μl were used for liquid scintillation counting in 5 ml of Bray's solution[10]. Roman numerals of the pooled fractions refer to the position of the corresponding peptides in the sequence of the B1-8.δ1 heavy chain as given in Fig. 2. Peptide no. III was insoluble in 6 M urea, 0.1 M formic acid, and could thus be isolated without chromatographic separation.